bio-protocol Preprint

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qRT-PCR

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An abbreviated version of this protocol was published in eLIFE in Mar 2020

Tumors attenuating the mitochondrial activity in T cells escape from PD-1 blockade therapy

DOI: 10.7554/eLife.52330

Detailed protocol

qRT-PCR protocol

- RNA was isolated with the RNeasy mini kit (QIAGEN, Hilden, Ger- many) and cDNA was synthesized by reverse transcription (Invitrogen).
- 1 ug of RNA was used for preparing cDNA.
- Further for qRT-PCR, we used 10ng of cDNA in 384 well format (10 ul reaction volume per well) for quantification. SYBR green protocol was followed for the qRT-PCR.

Step	Temp	Time	
Initial denaturation	94 °C	2 min	
Denaturation	94 °C	15 sec	x 55 cycles
Annealing, extension, and read fluorescence	60 °C	1 min	

- Relative quantification method used to compare the RNA quantity among different groups.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kumar, A. and Honjo, T. (2022). qRT-PCR. Bio-protocol Preprint. bio-protocol.org/prep1838.
2. Kumar, A., Chamoto, K., Chowdhury, P. S. and Honjo, T.(2020). Tumors attenuating the mitochondrial activity in T cells escape from PD-1 blockade therapy. eLIFE. DOI: 10.7554/eLife.52330

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